Molecular Technologies | Nucleic Acid Purification

Eppendorf® Prime RNase Inhibitor

Storage at –20°C in constant temperature freezer

Prime RNase Inhibitor is a protein of non-human origin that binds non-covalently and inhibits the same types of ribonuclease as HPRI, including RNases A, B, and C. Prime RNase Inhibitor can be used in cDNA synthesis, in in vitro transcription using the SP6, T7, and HeLa cell extract systems, and in in vitro translations using rabbit reticulocyte lysates and wheat germ extracts[1]. Additionally, Prime RNase Inhibitor prevents RNA degradation in cell extracts prepared under non-denaturing conditions[1], and it may be utilized in first strand synthesis prior to PCR[2] as well as in the RT-PCR of RNA isolated from single cells[3,4].

Prime RNase Inhibitor may also be used in gel retardation assays[5], the isolation of an RNA binding protein by oligonucleotide affinity purification[6], nuclear run-on transcription assays[7], in vitro transcription in S. cerevisiae whole cell extracts[8,9], and in polysome distribution analysis at 4°C. Prime RNase Inhibitor is most active in the absence of DTT.

Applications

- In vitro Transcription
- In vitro Translation
- First- and second-strand cDNA synthesis
- Preparation of RNA and mRNA I RT-PCR

Product features

- Inhibits greater than 90% of RNase activity, whereas Human Placental RNase Inhibitors (HPRI) inhibit only 50%
- Stable under a broad range of pH, DTT concentrations, and temperatures
- Inhibits RNases A, B, and C, similar to HPRI; does not interfere with RNases T1, T2, H, U1, U2, or CL3
- Sterile, DNase- and RNase-free

Quality Assurance

Each lot of Prime RNase Inhibitor contains no detectable RNase or DNase activity, as determined by overnight incubation of 30 units of Prime RNase Inhibitor with yeast RNA or plasmid DNA and I DNA-Hind III fragments, respectively, and subsequent analysis by agarose gel. Sterility is tested by plating 5 µl of inhibitor onto an LB-agar plate, followed by incubating at 37°C for 14–16 hours and then at room temperature for 14–24 hours. No growth is evident on the plate.

Storage Buffer

2.0 mM KH_2PO_4, 8.0 mM Na_2HPO_4, 3.0 mM KCl, 150 m[illegible] Glycerol.

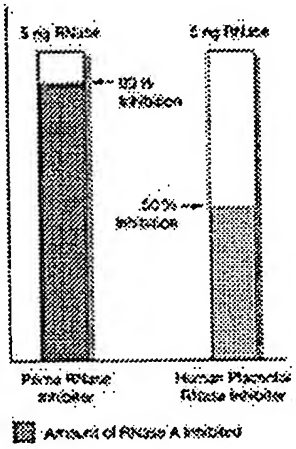
Concentration

30 units/µl.

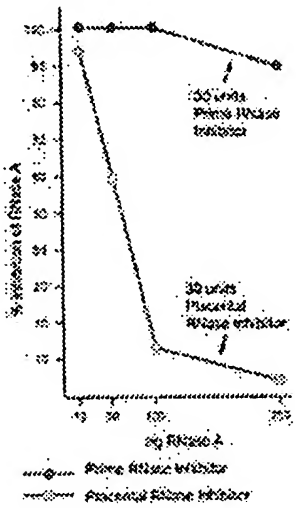
Units

30 units of the Prime RNase Inhibitor are required per 30 µl sample.

Amount of RNase Inhibitor



Potency of Prime RNase Inhibitor compared to HPRI



[1] Murphy, N. R., Leinbach, S. S., and Hellwig, R.J. BioTechniques 18(6): 1068–1073, 1995.

[2] Rother, R. P. BioTechniques 13(4): 524–527, 1992.

[3] Dulac, C., and Axel, R. Cell 83: 195–206, 1995.

[4] Berardi, A. C., Wang, A., Levine, J. D., Lopez, P., and Scadden, D.T. Science 267: 104–108, 1995.

[5] Hentze, M. W. et al. Science 244: 357–359, 1989.

[6] Rouault, T. A., et al. Proc. Natl. Acad. Sci. USA 86: 5768–5772, 1989.

[7] O'Conner, J. L. and Wade, M. F. BioTechniques 12(2): 238–243, 1992.

[8] Woontner, M. and Jaehning, J. A. J. Biol. Chem. 265: 8979–8982, 1990.

[9] Woontner, M., et al. Mol. Cell. Biol. 11: 4555–4560, 1991.